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Aseptic filling in Tetra Pak. Sterilization of the paper.

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ASEPTIC FILLING IN TETRA PAK STERILIZATION OF THE PAPER

(ASEPTISK TAPPNING I TETRA PAK
STERILISERING AV PAPPRET)

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Introduction.

In connection with the development of the Tetra Pak aseptic filling system one of the main bacteriological problems was to find a suitable method for the sterilization of the packaging material. Experiments for the purpose of solving this problem were started some five years ago at the research station in close co-operation with the staff of the section for technical development of the Tetra Pak Ltd. Most of the bacteriological work was carried out at Alnarp while the designing and manufacturing of the technical equipment was made at Tetra Pak. This paper is a report on the laboratory and semi-practical experiments. Results of full scale and commercial experiments have been reported elsewhere (3, 13.)

The packaging material of primary interest for the aseptic filling consists of a polyethylene (PE) coated kraft paper. The PE layer is applied on one side of the paper (the milk side) from an extruder at over 200° C. Consequently, the material is sterile on the milk side immediately after its manufacture. Afterwards, however, the paper is infected to a certain degree during rolling, printing, waxing, transport and handling.

The degree of infection of the ordinary commercial Tetra paper used for pasteurized milk was found to be of the magnitude of 0.02 bacteria per cm^2 with a rather uneven distribution of the bacteria. Such paper gave about 90 % sterile packages when used without previous sterilization for aseptic filling of sterile broth.

In commercial packaging of sterile milk the number of non-sterile packages cannot be allowed to exceed 1/1000 and should preferably be still lower. This means that the initial bacterial count on the PE surface of the Tetra paper must be reduced by at least 99.95-99.99 %. The time available for the bacterial reduction is limited to a certain extent by the design of the Tetra Pak machine and should not exceed 10—20 seconds.

In the search for an adequate sterilization technique various methods of investigation were used. The very low initial bacterial count on the PE surface and the necessary high killing effect made a direct determination of the sterilizing effect impossible. Therefore, the killing effect of the various agents was determined by use of artificially infected Tetra paper or, in the preliminary experiments, small glass plates. Spores of aerobic bacilli were used. The carrier of the spores was subjected to sterilization by the method to be tested. The bacterial count before and after this procedure was determined and the killing effect was calculated as a percentage of the initial count. The results obtained in this way were finally tested in a full scale aseptic Tetra Pak unit using sterile broth as a test fluid. In these experiments commercial paper without artificial infection was used.

From the list of sterilization procedures (see e. g. REDDISH, 10, MCCULLOCH, 7, SYKES, 14) possibly meeting the requirements, a few could be eliminated by fairly simple experiments. These procedures were sterilization by means of heat, ultra-violet radiation and high frequency electric fields. Sufficient heat treatment of the paper was found to make it brittle and difficult to seal. The other two methods were inefficient.

Sterilization by ethylene oxide gave promising results but was deemed technically inapplicable for the time being.

Other chemicals were tested. After promising preliminary results they were subjected to more detailed studies, which are reported here. One of the methods, involving the use of hydrogen peroxide and heat treatment, found technical application.

Material and methods.

Bacteria. Strains of *Bacillus cereus v. mycoides* and *Bacillus subtilis* were isolated from various sources or received from other laboratories. The resistance of the spores of the various strains to heat and hydrogen peroxide was tested by the Jensen cover glass method (see below). The strains showing resistance above average were selected for the investigations. These were *Bac. subtilis* 6002 (kindly provided by Prof. Lembke of Kiel) and *Bac. subtilis* 736 (kindly provided by the British National Collection of Dairy Organisms).

For the preparation of spore suspensions of these organisms the technique of HALVORSEN (2) was used. The spores were stored in a buffer solution as described by MORRISON and RETTGER (8).

Infection of the paper. A spore suspension in physiological saline was sprayed on to the PE surface of the paper. The density of the spore suspension and the spraying were such as to yield not more than ten colonies per cm^2 in the plate contact assay.

Pilot plant. A model sterilizing apparatus admitting the use of various liquid sterilizing agents and various drying and heating temperatures was manufactured. The principle of the apparatus is outlined in Figure 1. Figure 2 is a photographic picture.

The apparatus consisted of an aseptic chamber with an interchangeable sterilizer for the paper and a heating and a cooling device. This chamber was connected with an aseptic chamber with rubber arm gloves for the bacteriological sampling of the paper. The whole apparatus was kept under a slight superpressure of sterile filtered air.

The apparatus was sterilized by means of a solution of a quaternary ammo-

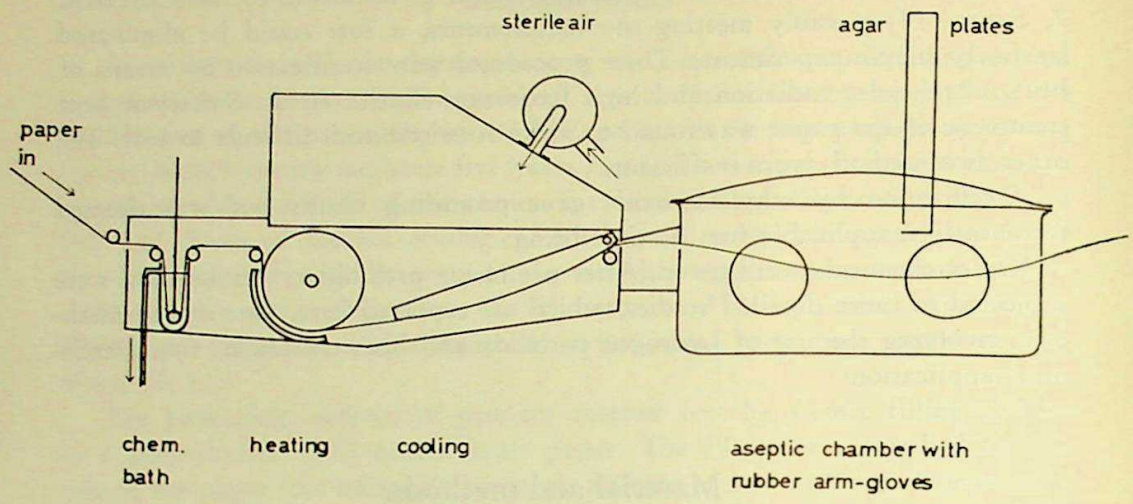

FIGURE 1. Model sterilizing apparatus arranged for a liquid sterilizer.

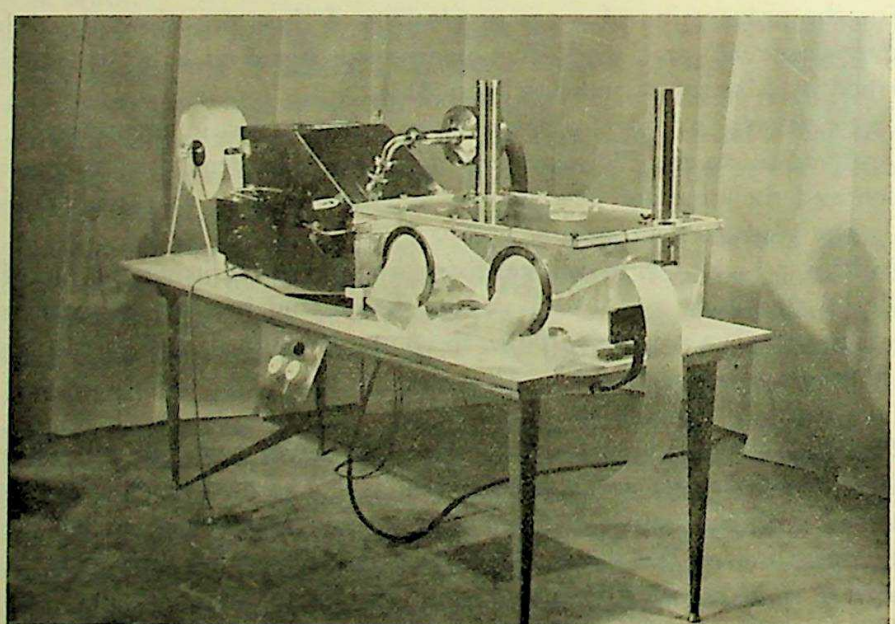

FIGURE 2. Model sterilizing apparatus.

nium compound. A slip of previously sterilized paper was attached to the artificially infected Tetra paper. The slip was inserted to the left, and was moved by an electric motor into the sampling section, passing the bath and the lifted heater and cooler. The chemical bath, the heater and the cooler were put into function. Swab-tests or plate contact assays were made in the last aseptic chamber. Finally, the chemical bath and the heating section were put out of action and the sampling for the determination of the "initial" count was made.

The speed of the paper was regulated so as to give a holding time in the bath and in the heating section of 11 seconds each. The temperature of the heating section could be kept constant at any level up to 250°C.

Large scale experiments. The large scale experiments were conducted by means of a convertible prototype Tetra Pak aseptic filling machine shown in Figures 3 and 4.

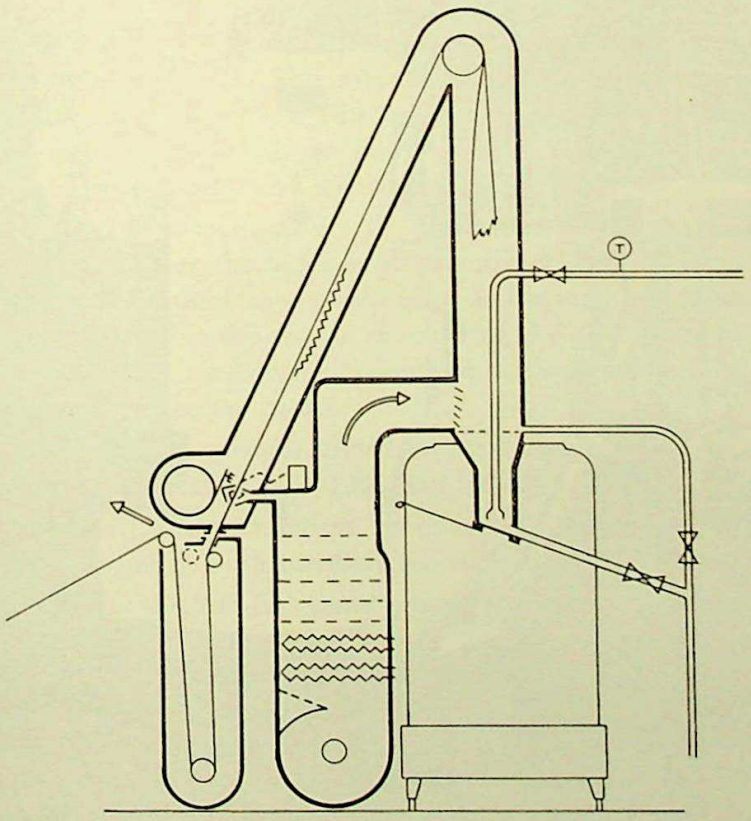

FIGURE 3. Convertible prototype Tetra Pak aseptic filling apparatus with chemical bath and heating elements.

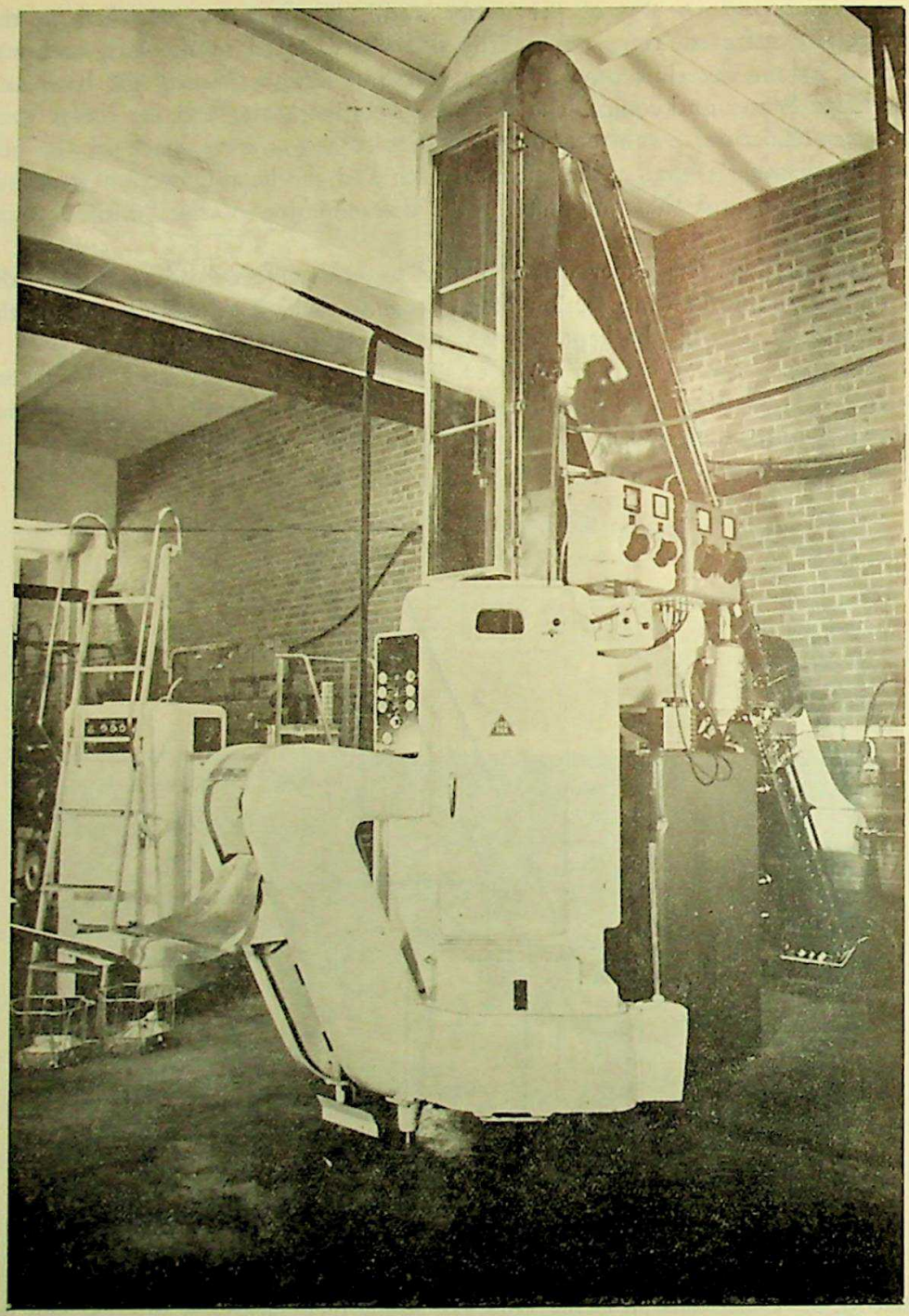

FIGURE 4. Convertible prototype Tetra Pak aseptic filling apparatus with chemical bath and heating elements.

This modification of the ordinary Tetra machine was equipped with a superstructure consisting of an aseptic channel enclosing the paper web. The channel was provided with an air blower, a sterile filter and a heating element for the air. Outside the channel a chemical bath or any other sterilizer could be attached.

After proper sterilization of the aseptic channel by hot air, the Tetra paper was moved through the chemical bath into the ascending part of the channel. An automatic device picked up the paper and fed it through the descending part and into the package-forming section of the machine. On the way through the ascending part the wet paper was heated and dried by means of infrared elements. The whole superstructure was under a slight superpressure of sterile filtered air. The holding time for the paper in the chemical bath was 11 seconds and the duration of the heating and drying about ten seconds.

In most of the technical experiments sterile broth was used instead of milk. The broth had the following composition: Bacto peptone 25 g, meat extract (Difco) 75 g, sodium chloride 12.5 g, glucose 20 g and water 10 litres.

In each experiment 300 kg broth was used. The ingredients were dissolved in a small amount of water. The solution was autoclaved and membrane filtered into a larger pressure tank filled with sterile water. The mixture was finally heated to 105°C for half an hour. Tubes, valves etc. were sterilized by steam, sodium hypochlorite and iodophores.

Bacteriological examination. In the pilot plant experiments the paper was investigated for the number of bacteria on the PE layer by means of the plate contact method as described by LINDQVIST and ANDERSON (6). The plates were prepared in the aseptic chamber of the pilot plant, a precaution necessary in view of the low bacterial counts to be determined. The incubation time was 72 hours and the temperature 30°C.

The bacterial density was calculated as an average value of about 100 agar plates covering a total area of 1200 to 1500 cm^2.

By the sterilizing effect is meant the number of bacteria killed in per cent of the initial number, both numbers referring to a unit area.

The plate contact method has some serious disadvantages. Thus there is a possibility that the agar plate does not pick up all bacteria from the surface investigated. Bacteria occurring in clusters or very close together will not show up as individual colonies on the agar and consequently the total count will tend to be too low. Comparative analyses of the artificially infected paper were made using the plate contact method and the swab contact technique (LABORATORY MANUAL, 5).The latter test gave 30—50 times higher counts than the plate method. The discrepancy would seem likely to be smaller in the determination

of the final count. If this is so, the sterilizing effect calculated on the basis of the plate contact counts would be too low, which would provide a certain margin of safety.

For preliminary experiments drops of the bacterial suspension were placed on small glass plates (5x5 mm). After drying, the glass plates were exposed to the sterilizing agent to be tested for a specified time. The plates were then immersed in sterile broth (meat extract peptone broth). After incubation for 72 hours the broth tubes were investigated for growth. The method has been described as Jensen's cover glass method (4). Experiments were also made with PE discs using the same technique.

In the large scale experiments the packages containing sterile broth were

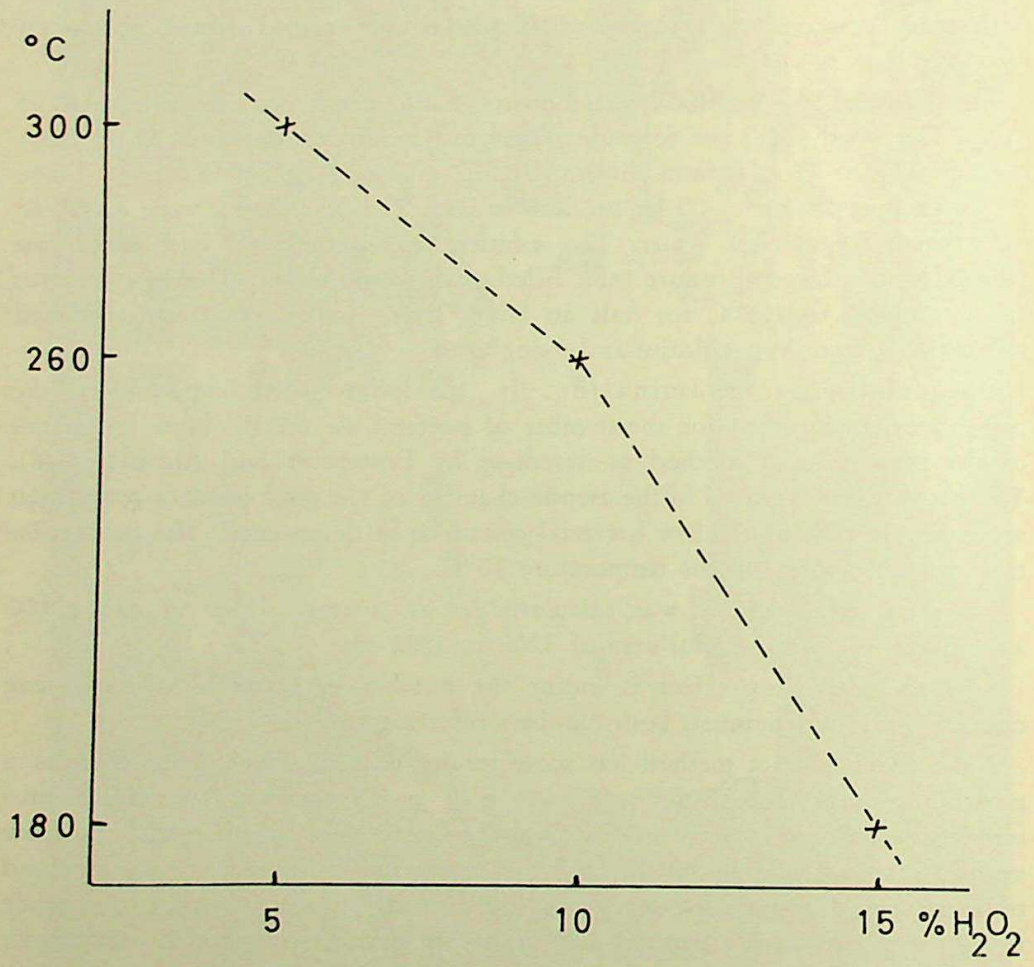

FIGURE 5. Minimum heating temperatures necessary to achieve complete killing of Bac. subtilis spores on glass plates exposed to hydrogen peroxide of various concentrations.

stored for seven days at 20 and 30°C. They were then opened and investigated turbidometrically for bacterial growth.

Results.

Hydrogen peroxide.

The preliminary experiments with the Jensen technique showed that hydrogen peroxide alone is not fast enough in its sporicidal action to ensure complete sterility in the maximal ten seconds available. Only when combined with a subsequent heat treatment for 8—10 secs (in hot air) the peroxide is sufficiently effective. The minimum requirements as regards concentration of the hydrogen peroxide and the temperature necessary to achieve 100 % killing effect in the Jensen technique are indicated in Figure 5 (page 8).

The temperatures necessary in these cases are too high for the PE coated paper to retain its properties. It was believed, however, that lower temperatures might be adequate though this could not be shown by the Jensen method. Experiments were therefore run in the pilot plant using different concentrations and a temperature treatment compatible with the properties of the paper. The results are summarized in Table 1.

Table 1. Sterilization of artificially infected Tetra paper with hydrogen peroxide and heat in pilot plant experiments.

Concentration of H_2O_2 %	Temperature in heating section	Initial count per cm^2	Sterilizing effect %
11	85	6.1	83
16	85	6.1	84
22	85	6.1	97
11	125	6.1	99.4
15	125	6.1	99.7
22	125	6.1	99.2

The highest sterilizing effect was achieved with the highest temperature chosen. This effect, though over 99 %, would seem too small for the sterilization of the commercial paper. However, taking into account the fact that the plate contact method might give too low values, the effect was thought likely to be sufficiently high. An effect of 99.98 % indeed, was found in separate experiments using the swab contact method after sterilization with 15 %

hydrogen peroxide and heating to 125°C.

This method for sterilizing the Tetra paper was adopted for practical experiments in the convertible prototype. In these experiments sterile broth was packed. In all 5500 packages were made in five batches and investigated for sterility. The results are given in Table 2.

Table 2. Large scale aseptic filling of sterile broth in the convertible prototype Tetra Pak. Hydrogen peroxide and heat.

Batch	Total no. of packages	Percentage sterile packages
1	345	98.5
2	1293	98.5
3	1302	99.8
4	1299	99.4
5	1320	99.0

Out of all packages 59 were found to be contaminated. If the first two batches are not included, the total sum would be 3900 packages. Out of these 23 or 0.6 % were contaminated.

Sodium hypochlorite.

The experiments concerning the efficiency of sodium hypochlorite were all made in the pilot plant. The factors studied were pH of the hypochlorite solution, its concentration, and the heating temperature. The results are given in Tables 3, 4 and 5.

Table 3. Influence of pH of hypochlorite solution on the sterilizing efficiency.

Available chlorine mg per l	pH of solution	Heating °C	Bacterial count per cm²	Sterilizing effect %
4000	11.0	none	2.9	19
4000	6.5	none	2.9	99.9
4000	5.5	none	2.9	99.8
4000	4.5	none	2.9	99.92

The pH of the sodium hypochlorite solution was adjusted by addition of hydrochloric acid. The sporicidal efficiency of the hypochlorite was greatly enhanced by the lowering of the pH of the solution.

The influence of the concentration of the hypochlorite on the sterilizing effect was studied at a pH of 4.5 in the solution. The results are recorded in Table 4.

Table 4. Influence of concentration of available chlorine on the sterilizing effect.

Available chlorine mg per l	pH of solution	Heating °C	Bacterial count per cm²	Sterilizing effect %
1000	4.5	85	3.5	99.94
2300	4.5	85	3.5	99.96
4000	4.5	85	3.5	99.98

The sporicidal effect of a solution of sodium hypochlorite with a concentration corresponding to 4000 mg available chlorine per litre at pH 4.5 would seem sufficient for practical use. A certain improvement might be achieved by the application of heat after the chlorine bath but is very difficult to demonstrate. Any possible differences between results at this high level of efficiency are very difficult to detect by the bacteriological methods used. Table 5 shows some results of experiments on the influence of heat treatment.

Table 5. Influence of heat treatment in connection with sterilizing by means of active chlorine.

Available chlorine mg per l	pH of solution	Heating °C	Bacterial count per cm²	Sterilizing effect %
4200	4.5	none	6.3	99.6
3900	4.5	85	3.5	99.96
3900	4.5	85	3.5	99.98
3900	4.5	180	6.3	99.98
3600	4.5	175	6.3	>99.99*)

*) no surviving microbe found in the investigated area.

There is some indication that even a moderate heat treatment of the paper increases the sporicidal efficiency of the sodium hypochlorite. In comparison with the effect achieved with hydrogen peroxide the acid hypochlorite solution would seem more suitable for the purpose.

Large scale experiments with hypochlorite had to be discontinued very early because of the corrosion of vital details in the Tetra machine caused by the sodium chloride present in the paper after the drying.

Chlorine dioxide.

Owing to the difficulties bound up with the technical application of hypochlorite sterilization of the paper, attention should be paid to other chlorine compounds, preferably those giving no residue in the paper after the drying. Chlorine dioxide was chosen for investigation. For information concerning chlorine dioxide as a sterilizing agent see references 1, 9, 11 and 12. Degussa Ltd., Frankfurt am Main, supplied information for the synthesis of this compound for the experiments.

A solution of sodium chlorite ($NaClO_2$) was added slowly to dilute hydrochloric acid. The chlorine dioxide formed was transferred continuously from the reaction mixture into distilled water by means of a stream of nitrogen. The concentration was determined titrimetrically.

The aqueous solution of chlorine dioxide was used as a sterilizing agent in pilot plant experiments. The results obtained are recorded in Table 6.

Table 6. Sterilizing efficiency of a solution chlorine dioxide in water.

ClO_2 mg per l		Heating °C	Bacterial count per cm^2	Sterilizing efficiency %
before	after			
350	—	85	6	99.32
400	325	85	6	97.65
550	400	125	6	99.32

The results compare fairly well with those obtained with hydrogen peroxide. The high volatility of the chlorine dioxide makes it necessary to add the active compound continuously to the solution during longer experiments or in practical application. The concentration seems to have to be kept at about 500 mg chlorine dioxide per litre with a subsequent moderate heat treatment of about 125°C.

Large scale experiments have yet to be performed.

Summary.

Various sterilization methods for the paper were investigated in connection with the development of the Tetra Pak aseptic filling system.

Treatment of the paper with a 15—20 % solution of hydrogen peroxide followed by moderate heating was found to meet the theoretical and practical requirements.

Sodium hypochlorite, 4000 mg available chlorine per litre at pH 4.5, was

found to be still more effective. Difficulties due to corrosion have so far prevented technical application.

Chlorine dioxide solution of about 500 mg per litre was shown to be sufficiently effective. Practical experiments with this chemical have yet to be performed.

SAMMANFATTNING

Ett av huvudproblemen vid utvecklandet av den aseptiska förpackningen i Tetra Pak var att finna en lämplig metod för sterilisering av förpackningsmaterialet. Försök att lösa detta problem startades för fem år sedan vid Statens mejeriförsök i nära samarbete med utvecklingsavdelningen vid Tetra Pak. Det bakteriologiska arbetet utfördes i Alnarp, medan konstruktion och tillverkning av försöksapparatur skedde vid Tetra Pak.

Det mest aktuella förpackningsmaterialet utgöres av polyetylenbelagt kraftpapper. Omedelbart efter polyetylenbeläggningen är materialet sterilt, men efterföljande behandling medför en infektion som i genomsnitt uppgår till ca 0,02 bakterier per cm^2. Bakterierna är ojämnt fördelade, så att sådant papper, om det icke steriliseras, vid aseptisk förpackning ger ca 90 % sterila paket.

Vid kommersiell förpackning av steril mjölk får högst en paket per 1000 vara osteril. Detta innebär, att bakterieantalet på det ordinära Tetra-pappret måste nedbringas med 99,95—99,99 %. Åtskilliga tänkbara steriliseringsmetoder prövades i preliminära experiment. Vid försöken användes dels Jensens täckglasmetod, dels en för ändamålet utvecklad försöksteknik. I denna utsattes papper, som infekterades med sporer av *Bacillus subtilis* för ett steriliserande medel. Med svabb- eller tryckplattemetoden räknades bakterieantalet före och efter behandlingen och steriliseringseffekten beräknades i procent av begynnelsebakterietalet.

Värme, ultraviolett ljus och högfrekvent elektriskt fält visade sig icke vara användbara steriliseringsmetoder. Tillräcklig värmebehandling gjorde pappret skört och svårt att försegla. De övriga metoderna var icke effektiva. Etylenoxid var tillräckligt effektiv, men metoden bedömdes vara opraktisk i detta sammanhang. Behandling av pappret i kemiskt bad med 15—20 %-ig vätesuperoxid följd av upphettning och torkning visade sig ge över 99,9 % i steriliseringseffekt. Vid förpackning i stor skala av steril buljong var i olika försök 99,5—99,8 % av paketen sterila. Denna metod har fått praktisk användning.

Aktiv klor i form av en lösning av natriumhypoklorit, 4000 mg aktiv klor per l vid pH 4,5, gav likaledes efter värmning av pappret över 99,9 % steri-

litet. Korrosionssvårigheter framtvang avbrytande av försöken. Försök i liten skala med en vattenlösning av klordioxid (500 mg per l) gav goda resultat. Försök i stor skala med klordioxid har ännu icke utförts.

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